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Shilpa Parashuram
ICAR-National Research Centre on Pomegranate, Solapur, Maharashtra, India

NV Singh
ICAR-National Research Centre on Pomegranate, Solapur, Maharashtra, India

P Roopasowjanya
ICAR-National Research Centre on Pomegranate, Solapur, Maharashtra, India

KD Babu
ICAR-National Research Centre on Pomegranate, Solapur, Maharashtra, India

Vipul Sangnure
ICAR-National Research Centre on Pomegranate, Solapur, Maharashtra, India

Sarvesh Singh
ITM University, Gwalior, M.P., India

Jyotsana Sharma
ICAR-National Research Centre on Pomegranate, Solapur, Maharashtra, India

RK Pal
ICAR-National Research Centre on Pomegranate, Solapur, Maharashtra, India

Correspondence
Shilpa Parashuram
ICAR-National Research Centre on Pomegranate, Solapur, Maharashtra, India

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Germplasm conservation and phenotypic characterization of pomegranate (*Punica granatum* L.) germplasm accessions for various morphological and physico-chemical characters

Shilpa Parashuram, NV Singh, P Roopasowjanya, KD Babu, Vipul Sangnure, Sarvesh Singh, Jyotsana Sharma and RK Pal

Abstract
Pomegranate (*Punica granatum*L.), a rapidly emerging commercial fruit crop of India to meet the domestic requirements as well as export demands. It is widely cultivated in India from Jammu & Kashmir to Karnataka, due to its adaptability to the diverse climatic conditions, less natural resource demanding nature and high return on investment. Pomegranate is also an ideal fruit crop for utilizing sub-marginal and degraded lands having pH as high as 8.5 provided there is good drainage. With the need of developing improved cultivars over the existing ones, ICAR-National Research Centre on Pomegranate, Solapur, has collected, conserved and maintained over 350 diverse pomegranate germplasm accessions at its Field Gene Banks. These pomegranate germplasm at Field Gene Banks are maintained with minimum tillage of tree beds following principles of conservation agriculture using brush cutter. The effective utilization of these germplasm resources will be possible only after characterization for each of the desirable characters. In the present study, 25 pomegranate accessions were evaluated for 25 quantitative traits in Randomized Block Design with three replications in *mrig bahar* of 2016-17. The recorded data has showed the presence of variability for the studied morphological and physico-chemical traits. In ANOVA, significant differences between the germplasm accessions were observed at both 5% and 1% level of significance, indicating adequate variability among the genotypes for all characters. Mean performance of the genotypes for fruit yield attributing traits was observed to be 80.25-207.10g (fruit weight), 4.81-6.61cm (fruit length), 4.94-7.08cm(fruit diameter), 18.77-45.23 g (100 arils weight), 26.65-122.05g(total arils weight), 34.93-82.90g(rind weight), 1.83-3.69 mm (Rind thickness), 8.58-11.4 mm(Aril length), 5.4-7.76 mm(Aril width), 15.86-19.2^{0}Brix(TSS), 1.51-3.42% (Acidity), 26.01-52.02%(Fruit Juiciness), 68.4-109.92N (Seed texture).Among all traits, total arils weight(43.55) has showed highest Coefficient of Variation (%) value followed by fruit weight (33.75), rind weight (31.53) and 100 arils weight (29.77). Depending on the breeding objectives, selection can be made among these 25 genotypes for traits of interest to develop improved varieties in pomegranate.

Keywords: Pomegranate, germplasm accessions, quantitative traits, randomized block design, coefficient of variation.

Introduction
Pomegranate (*Punica granatum* L.), a rapidly emerging commercial fruit crop of India which is also an ideal fruit crop for utilizing sub-marginal and degraded lands having pH as high as 8.5 provided there is good drainage. Pomegranate cultivation is a highly lucrative and remunerative agriculture business in India. The adaptability to a wide range of climatic conditions, hardy nature, low water requirement, good response to high tech-horticultural practices, high yield, returns on investment higher than many crops of dry regions, immense therapeutic values and high export potential has made pomegranate a popular fruit of tropical and subtropical regions in recent times (Sharma *et al*., 2014) [12].

It is widely cultivated throughout India, Iran, China, Turkey, USA, Spain, Azerbaijan, Armenia, Afghanistan, Uzbekistan, the Middle East, Pakistan, Tunisia, Israel, dry regions of Southeast Asia, Peninsular Malaysia, the East Indies and tropical Africa (Sharma *et al*., 2014) [12]. India is the world leader in pomegranate acreage and production with 208.73 thousand ha area and 2442.39 thousand tones of annual production (http://nhb.gov.in, Horticultural Statistics at a Glance, 2017, GoI). In India, pomegranate is extensively grown in Maharashtra,

Karnataka, Andhra Pradesh, Gujarat, Telangana and is picking up fast in, Himachal Pradesh, Rajasthan and Madhya Pradesh. Small areas are under cultivation in Tamil Nadu, Mizoram, Odissa, Nagaland, Lakshadweep, Jharkhand and Jammu and Kashmir (Sharma *et al*., 2014) [12].

The North Himalayas of India has rich diversity of pomegranate where wild type seedling trees locally known as *Daru* come up naturally in abundance. The fruits of such genotypes are highly acidic and commercially used for preparation of *anardana* (dried arils), these wild types are highly vigorous and are source of moderate resistance to bacterial blight disease and other pathogens (Jalikop *et al*., 2005; Singh *et al*., 2008; Chandra *et al*., 2013) [6, 13, 3]. Sweet type pomegranate germplasm are available in plenty in different parts of India and have wide variations in their fruit characters (Khodade *et al*., 1990; Chandra *et al*., 2011) [7, 2]. With the need of developing improved cultivars over the existing ones, ICAR-National Research Centre on Pomegranate, Solapur, has collected, conserved and maintained over 350 diverse pomegranate germplasm accessions at its Field Gene Banks. These pomegranate germplasm at Field Gene Banks are maintained with minimum tillage of tree beds following principles of conservation agriculture using brush cutter. The effective utilization of these germplasm resources will be possible only after characterization for each of the desirable characters. In the present study, 25 pomegranate accessions were evaluated for 25 quantitative traits in Randomized Block Design with three replications in *mrig bahar* of 2016-17.

Materials and Methods

Experimental site and climatic conditions

The study was carried out during the period of 2016-17 at B6 and C1 blocks, Kegaon Research Farm of the ICAR-National Research Centre on Pomegranate, Solapur, India, located at 17°43' N latitude, 75°50' E longitude and 475 m altitude from mean sea level. The average relative humidity for the entire growing season of both the years ranged between 85.35 to 56.26 and the average maximum and minimum temperature were 33.66 and 19.94 °C, respectively.

Pomegranate genotypes

Twenty five pomegranate accessions were selected from the field gene banks of ICAR-National Research Centre on Pomegranate, Solapur (Table 1). These accessions were evaluated for 25 various quantitative traits in Randomized Block Design with three replications in *mrigbahar* of 2016-17.

Table 1: List of pomegranate genotypes evaluated for various morphological, physico-chemical characters

Sl. No.	Genotype name	Source	Country
1	IC318703	Shimla	India
2	IC-318706	Shimla	India
3	IC-318718	Shimla	India
4	IC-318720	Shimla	India
5	IC-318723	Shimla	India
6	IC-318724	Shimla	India
7	IC-318728	Shimla	India
8	IC-318734	Shimla	India
9	IC-318735	Shimla	India
10	IC-318743	Shimla	India
11	IC-318744	Shimla	India
12	IC-318762	Shimla	India
13	IC-318766	Shimla	India
14	IC-524027	Bhowali	India
15	IC-524028	Bhowali	India
16	IC-524030	Bhowali	India
17	IC-524031	Bhowali	India
18	IC-444199	Bhowali	India
19	IC-444200	Bhowali	India
20	IC-444201	Bhowali	India
21	IC-444204	Bhowali	India
22	IC-444206	Bhowali	India
23	IC-444208	Bhowali	India
24	IC-0599595	Jammu & Kashmir	India
25	IC-0599597	Jammu & Kashmir	India

Physical characteristics of fruits and trees

Twenty five various morphological and physico-chemical quantitative traits were evaluated according to the PPV& FRA descriptors developed for pomegranate. The traits considered were : tree height (m), tree spread (m), leaf blade length (cm), leaf blade width(cm), calyx length (mm), calyx width (mm), petal length (mm), petal width (mm), fruit weight (g), fruit length (cm), fruit diameter (cm), no. of arils/fruit, 100 arils weight (g), total arils weight (g), rind weight (g), rind thickness (mm), aril length (mm), aril width (mm), total soluble solids (0Brix), titrable acidity (%), fruit Juiciness (%), 100 seed weight (g), seed length (mm), seed width (mm), seed texture (N).

For each accession, three trees were considered for morphological characterization. For each tree, samples of five mature leaves, five hermaphrodite and five fully maturefruits harvested were studied for various quantitative traits during *mrig bahar* of 2016-17 (Table 2). The titrable acidity (TA) was determined by titration against 0.1 N NaOH solution and expressed in terms of gram citric acid per 100ml of juice (Ranganna, S. 2001) [10]. The total soluble solids (TSS) were determined using a digital refractometer (model SMART-1, ATAGO, Tokyo) and reported as °B at 21 °C.

Statistical Analysis

The recorded data was subjected to Analysis of variance

(ANOVA) to test the significant differences between the genotypes. Genotypic divergence between the genotypes was assessed by Mahalanobis D^2 statistic by using GENRES software (Mahalanobis, 1936) [8]. On the basis of D^2 values, the genotypes weregrouped into different clusters as suggested by Tocber (Rao, 1952) [11].

Results and Discussion

Various morphological and physico-chemical properties data showed a significant genetic variability among 25 genotypes for the given characteristics at both 5% and 1% level of significance in ANOVA (Table 2). Mean, Range, Standard error of mean, and coefficients of variation values calculated for the studied 25 traits are reported in Table 2. The maximum plant height and spread were varied from 2.93 (IC-0599597) -4.30 (IC-444200) m and 3.40 (IC-318724)-4. 81(IC-524030) m. Leaf characters have shown mean values of coefficient of variation between the ranges of 12.16-12.77%. IC-444204accession was found to have the shortest (3.73 cm) leaves, while IC-IC-318706 accession had broadest (1.66 cm) leaves. Regarding flower characters, the calyx length and width of hermaphrodite flowers varied between 25.63 (IC-524028)-36.72 (IC-318728) mm and 7.69 (IC-0599595)-12.65 (IC-318762) mm (Table 3).

Fruit weight varied between a minimum of 80.25g found in IC-444200 and a maximum of 207.1g inIC-318706with an average weight of 132.03g and a high coefficient of variation (33.75%). Fruit length and diameter differed with the range value of 4.81(IC-318766) -6.61(IC318703) cm and 4.94(IC-318766) -7.08 (IC-318706)cm. Rind weight varied between 11 34.93g inIC-444204and 82.9g in IC-318706with an average of55.17 g and a coefficient of variation of 31.53%. Number of arils per fruit varied from 127.5 as seen in IC-318766to 322.93in IC-444208. Rind thickness also varied among genotypes with a minimum of 1.83 mm in IC-318734and a maximum of3.69mm in IC-524031 (Table 3).

Among the studied accessions the fruit Juiciness ranged from 26.01 (IC-318766)-52.02 (IC318703) %. Juice of the studied all genotypes were found to be acidic in nature with the maximum and minimum value of 1.51% (IC-444199) and 3.42 (IC-318718) %, while the total soluble solids (TSS) was found to be varied between 15.86 (IC-524028)-19.2 (IC-318744) ^{0} Brix. Hence these genotypes will be more suitable for anardana purpose. The average aril length and width was found to be 9.83mm and 6.58mm with the coefficient of variation of 10.68% and 10.66%. The coefficient of variation for seed characters was found to be in the range of 6.7 (seed length) %-12.09 (seed texture) % (Table 3). As seeds of all the genotypes were found to be hard with the range of 68.4 (1205)-109.92 (1185) N, will not be preferable for table purpose. Similar significant genetic variation in plant, leaf, flower and fruit characteristics have been reported in India (Chandra *et al*., 2014) [12], Turkey (Caliskan and Bayazit, 2013) [1], Pakistan (Nafees *et al.,* 2015) [9], Iran (Zarei 2017) [14], Lebanon (Dandachi *et al*., 2017) [5].

Table 2: Analysis of variance of morphological, physico-chemical characteristics of the studied pomegranate genotypes

SOV	df	TH (m)	TS (m)	LBL (cm)	LBW (cm)	CL (mm)	CW (mm)	PL (mm)	PW (mm)	FW (g)	FL (cm)	FD (cm)	NAF
Genotypes	24	0.35**	0.43**	1.09**	0.08**	36.06**	7.27**	38.68**	14.13**	4765.93**	1.05**	1.31**	6222.16**
Error	48	0.07	0.09	0.08	0.01	2.92	0.41	1.01	0.99	595.35	0.13	0.17	1784.25

SOV	df	100 AW (g)	AW (g)	RW (g)	RT (mm)	AL (mm)	AW (mm)	TSS (0Brix)	A (%)	FJ (%)	100 SW (g)	SL (mm)	SW (mm)	ST (N)
Genotypes	24	238.68**	2688.34**	645.18**	0.79**	2.77**	0.92**	3.07**	0.72**	166.29**	0.32**	0.39**	0.16**	244.87**
Error	48	5.12	153.97	131.38	0.10	0.27	0.28	0.30	0.11	6.96	0.00	0.07	0.03	29.12

Table 3: Mean, range and coefficients of variation for 25 quantitative characters of pomegranate accessions

Characters	Mean	Range	SEm±	Coefficient of variation (%)
Tree height (m)	3.52	2.93-4.3	0.16	11.58
Tree spread (m)	3.97	3.4-4.81	0.17	11.4
Leaf blade length (cm)	5.05	3.73-6.06	0.16	12.77
Leaf blade width(cm)	1.44	1.09-1.66	0.05	12.16
Calyx length (mm)	30.76	25.63-36.72	0.99	12.15
Calyx width (mm)	10.26	7.69-12.65	0.37	16.01
Petal length (mm)	18.51	13.18-24.49	0.58	19.89
Petal width (mm)	12.75	8.8-16.83	0.57	18.18
Fruit weight (g)	132.03	80.25-207.10	14.09	33.75
Fruit length (cm)	5.72	4.81-6.61	0.21	11.56
Fruit Diameter (cm)	6.04	4.94-7.08	0.24	12.24
No. of Arils/fruit	234.07	127.5-322.93	24.39	24.41
100 arils weight (g)	30.6	18.77-45.23	1.31	29.77
All arils weight (g)	72.57	26.65-122.05	7.16	43.55
Rind weight (g)	55.17	34.93-82.9	6.62	31.53
Rind thickness (mm)	2.6	1.83-3.69	0.19	22.17
Aril length (mm)	9.83	8.58-11.4	0.3	10.68
Aril width (mm)	6.58	5.4-7.76	0.3	10.66
TSS (ºBrix)	17.36	15.86-19.2	0.31	6.37
Acidity (%)	2.44	1.51-3.42	0.19	22.79
Fruit Juiciness (%)	37.97	26.01-52.02	1.52	20.41
100 seed weight (g)	2.3	1.81-2.73	0.03	14.46
Seed length (mm)	6.26	5.47-7.22	0.15	6.7
Seed width (mm)	2.67	2.36-3.18	0.1	10.13
Seed texture (N)	83.11	68.4-109.92	3.12	12.09

On the basis of magnitude of D^2 value, 25 genetically diverse genotypes were grouped into nine clusters (Table 4). The highest numbers of genotypes were presented in **Cluster II** (1196, IC-318718, 1182, 1181, 1197) and **Cluster-V** (IC-318766, 1201, 1198, 1203, IC-318735) with five genotypes in each followed by **Cluster- I** (IC-318743, IC318703, IC-318720) and **Cluster-III** (IC-318723, IC-318706, IC-318762) which contained three entries each, other Clusters had two genotypes each (**Cluster IV** -IC-318734, IC-318724; **Cluster VI**-1184, Acc. No 5; **Cluster VII**- Acc. No 2, IC-318744; **Cluster VIII**-1205, 1185). **Cluster-IX** was found to be solitary cluster with single genotype (IC-318728).

The highest intra-cluster distance was observed in case of **Cluster- I** (18.36), followed by **Cluster-V**(17.80) and **Cluster-VIII**(17.71). The maximum inter cluster distance was found between **Cluster-IV** and **Cluster-VIII** (35.04), followed by **Cluster-II** and **Cluster-IV** (33.04). Thus, crossing between the genotypes belonging to cluster pair separated by very high inter-cluster distances, will produce desirable transgressive segregates with an ample opportunity for selecting better genotypes in succeeding generations.

The clustermeans of the various plant, leaf, flower and fruitcharacters are presented in Table 5. The average clustermeans revealed highest values for the characters like No. of Arils/fruit (241.24), Fruit weight (166.12g), All arils weight (100.65g), Seed texture (84.04N), Rind weight (62.25g), Fruit Juiciness (43.44%) in Cluster I. Similar trend was observed for the other clusters also.

The per cent contribution of different morphological and physico-chemical characters towards genetic divergence was ranged from 0.00 % to 43.67%. Average fruit weight (g) contributed maximum (43.67%), followed by 100 seed weight (g) (30.33%) towards genetic divergence among these 25 germplasm accessions (Table 6).

Table 4: Intra and inter cluster distance (D^2).

Cluster	I	II	III	IV	V	VI	VII	VIII	IX
I	18.36	29.74	13.19	14.05	28.90	28.18	23.10	30.54	12.54
II		13.68	30.66	33.04	15.55	15.33	16.19	15.12	31.98
III			9.28	8.54	29.78	29.74	23.31	31.97	10.09
IV				8.04	32.18	32.55	26.08	35.04	9.69
V					17.80	17.00	16.34	16.64	31.32
VI						11.19	14.30	13.97	30.05
VII							13.37	16.83	25.49
VIII								17.71	32.93
IX									0

Table 5: Cluster means of 25 pomegranate genotypes

Character	Clusters								
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**
Tree height (m)	3.21	3.95	3.26	3.33	3.61	3.48	3.40	3.52	3.27
Tree spread (m)	3.77	4.15	3.61	3.64	4.10	4.48	3.99	4.09	3.41
Leaf blade length (cm)	5.01	5.04	5.49	5.67	4.51	5.37	5.31	4.63	5.01
Leaf blade width(cm)	1.48	1.41	1.45	1.48	1.32	1.63	1.55	1.35	1.61
Calyx length (mm)	32.67	28.99	34.55	34.00	28.40	30.91	29.70	27.21	36.72
Calyx width (mm)	11.26	9.34	12.19	12.11	10.04	9.13	8.93	8.53	11.92
Petal length (mm)	20.62	16.11	23.24	23.56	16.84	15.42	18.81	14.28	22.36
Petal width (mm)	13.80	11.12	15.19	15.66	12.07	10.95	12.67	11.12	15.02
Fruit weight (g)	166.12	97.50	180.94	178.22	102.48	124.73	100.03	143.53	166.75
Fruit length (cm)	6.17	5.25	6.30	6.32	5.27	5.85	5.25	5.90	6.32
Fruit Diameter (cm)	6.56	5.54	6.84	6.71	5.46	6.00	5.63	6.31	6.67
No. of Arils/fruit	241.24	230.17	262.60	276.13	207.89	211.03	203.07	270.83	227.87
100 arils weight (g)	40.35	22.70	41.67	41.03	23.53	25.85	26.45	27.95	45.23
All arils weight (g)	100.65	50.85	108.86	113.03	49.61	53.52	49.95	72.13	106.13
Rind weight (g)	62.25	43.50	67.90	60.58	47.08	63.89	47.04	66.94	59.07
Rind thickness (mm)	2.29	2.55	2.33	2.00	2.67	3.25	2.98	3.20	2.16
Aril length (mm)	10.82	8.83	10.98	11.05	9.23	9.14	9.50	9.68	11.40
Aril width (mm)	6.82	6.11	7.46	7.19	6.11	6.51	6.41	6.79	6.81
TSS (ºBrix)	17.65	16.99	18.09	17.52	17.52	16.17	17.64	17.12	17.37
Acidity (%)	2.19	2.52	2.16	2.35	2.54	3.00	2.82	2.02	2.31
Fruit Juiciness (%)	43.44	34.86	44.16	48.97	35.18	29.80	34.00	32.06	46.71
100 seed weight (g)	2.65	1.94	2.67	2.70	2.17	2.11	2.28	2.04	2.65
Seed length (mm)	6.40	5.91	6.67	6.62	6.20	6.10	6.53	5.89	6.39
Seed width (mm)	2.64	2.82	2.76	2.62	2.60	2.56	2.56	2.77	2.48
Seed texture (N)	84.04	77.90	85.02	82.40	85.10	79.59	81.91	89.16	89.52

Table 6: Contribution of each character to divergence

Names of characters	No. of first rank	% Contribution
Tree height (m)	0	0
Tree spread (m)	1	0.33
Leaf blade length (cm)	0	0
Leaf blade width(cm)	4	1.33
Calyx length (mm)	0	0
Calyx width (mm)	1	0.33
Petal length (mm)	2	0.67
Petal width (mm)	1	0.33
Fruit weight (g)	131	43.67
Fruit length (cm)	1	0.33
Fruit Diameter (cm)	0	0
No. of Arils/fruit	0	0
100 arils weight (g)	3	1
All arils weight (g)	1	0.33
Rind weight (g)	7	2.33
Rind thickness (mm)	0	0
Aril length (mm)	0	0
Aril width (mm)	0	0
TSS (0Brix)	4	1.33
Acidity (%)	4	1.33
Fruit Juiciness (%)	26	8.67
100 seed weight (g)	91	30.33
Seed length (mm)	8	2.67
Seed width (mm)	14	4.67
Seed texture (N)	1	0.33

Conclusion

A significant diversity in selected pomegranate genotypes was found based on the present evaluation of morphological and physico-chemical characteristics. Among fruit characteristics, the most widely varied traits were total arils weight, fruit weight, rind weight, and 100 arils weight with coefficients of variation of 43.55, 33.75, 31.53, and 29.77%, respectively. All the genotypes found suitable for anardana purpose as they are acidic in nature. The genetic diversity study has revealed that the fruit weight (g) and100 seed weight (g) traits have contributed for the genetic divergence among these accessions. As these traits are quantitative in nature needs to be evaluated for one more year along with the assistance of molecular markers.

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